Towards the preparation of novel Re/99mTc Tricarbonyl-containing peptide nucleic acid bioconjugates

Gasser, Gilles ; Sosniak, Anna M ; Leonidova, Anna ; Braband, Henrik ; Metzler-Nolte, Nils

Abstract: A novel azido derivative of the di-(2-picolyl)amide (Dpam) ligand, namely 3-azido-N,N-bis-pyridin-2-ylmethyl-propionamide (3), was prepared from 3-bromo-N,N-bis(pyridin-2-ylmethyl)propanamide (2) with an excess of sodium azide in DMSO. 3 was then reacted, by CuI-catalyzed [3 + 2] cycloaddition (often referred to as 'Click Chemistry'), with the previously reported alkyne-containing peptide nucleic acid (PNA) monomer Fmoc-1-OtBu to give the Dpam-containing PNA monomer (Fmoc-4-OtBu) in 44% yield. It was also demonstrated that 3 could be reacted by Click Chemistry, on the solid phase, to an alkyne-containing PNA oligomer (Alkyne-PNA) to yield Dpam-PNA. Our attempts to complex Dpam-PNA with [NEt4]2[ReBr3(CO)3] and [99mTc(CO)3(H2O)3]+ are also discussed in detail.

DOI: https://doi.org/10.1071/CH11010

Towards the Preparation of novel Re/^{99m}Tc Tricarbonyl-Containing Peptide Nucleic Acid Bioconjugates

Gilles Gasser,[A,*] *Anna M. Sosniak,*[B] *Anna Leonidova,*[A] *Henrik Braband,*[A] *and Nils Metzler-Nolte*[B,*]

[A] Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

[B] Ruhr-University Bochum, Faculty of Chemistry and Biochemistry, Bioinorganic Chemistry Department I, Universitätsstrasse 150, D-44801 Bochum, Germany.

Keywords: Peptide Nucleic Acid (PNA), Bioorganometallics, Organometallic Chemistry, Rhenium Compounds, Technetium Compounds, Click Chemistry, Radiolabelling.

Abbreviations: Boc - *tert*-butoxycarbonyl; Dpa - di-(2-picolyl)amine; Dpam - di-(2-picolyl)amide; ESI-MS - electrospray ionisation mass spectrometry; Fmoc – fluorenylmethoxycarbonyl; MALDI-TOF – matrix assisted laser/desorption ionization - time of flight; PNA – peptide nucleic acid; SPPS – solid phase peptide synthesis; TFA – trifluoroacetic acid.

[*] Corresponding authors: email: gilles.gasser@aci.uzh.ch; Phone: +41 44 635 46 11. Fax: +41 44 635 68 03; WWW: www.gassergroup.com. Email: nils.metzler-nolte@rub.de; Phone: +49 234 322 8152; Fax: +49 234 321 4378 ; WWW : www.rub.de/ac1.

Abstract

A novel azido derivative of the di-(2-picolyl)amide (Dpam) ligand, namely 3-azido-*N,N*-bis-pyridin-2-ylmethyl-propionamide (**3**), was prepared from 3-bromo-*N,N*-bis(pyridin-2-ylmethyl)propanamide (**2**) with an excess of sodium azide in DMSO. **3** was then reacted, by by Cu(I)-catalysed [3+2] cycloaddition (often referred as "Click Chemistry"), with the previously reported alkyne-containing Peptide Nucleic Acid (PNA) monomer **Fmoc-1-O^{t}Bu** to give the Dpam-containing PNA monomer" (**Fmoc-4-O^{t}Bu**) in 44% yield. It was also demonstrated that **3** could be reacted by Click Chemistry, on the solid phase, to an alkyne-containing PNA oligomer (**Alkyne-PNA**) to yield **Dpam-PNA.** Our attempts to complex **Dpam-PNA** with $[NEt_4]_2[ReBr_3(CO)_3]$ and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ are also discussed in detail.

Introduction

Peptide nucleic acids (PNAs) are unnatural DNA analogues, which have been discovered nearly 20 years ago.[1,2] Their uncharged pseudopeptide backbone is made of *N*-(2-aminoethyl)glycine units which are linked via a methylene carbonyl to the four DNA nucleobases.[1,3-5] PNAs has been investigated in various fields of research such as antisense[6] and antigene[7] therapies, specific target-directed cellular and *in vivo* gene repair[8] and biosensing.[9] Moreover, there is a growing interest on the preparation of (multi-)metal-containing PNA monomers/oligomers.[10-18] These "metal" modifications are usually performed to add new functionalities and/or spectroscopic properties to PNAs.[19-22] For example, ferrocene or rhenium derivatives have been conjugated to PNA sequences for electrochemical biosensing and fluorescence purposes, respectively.[17,23]

From a more synthetic point of view, the metal complexes have been, up to very recently, mainly attached to the *N*-terminus of the PNA sequence or to a side-chain of an amino acid (usually the amino group of lysine), limiting therefore the possibility of positioning a metal complex at any site within the PNA oligomer. However, our groups have lately presented a general approach to modify PNA oligomers with metal complexes using the Cu(I)-catalysed [3+2] cycloaddition,[24,25] which is often referred as "Click Chemistry".[10] In these reports, we have demonstrated that an alkyne-modified PNA monomer[10] (**Fmoc-1-OH**, Figure 1) could be inserted anywhere within a PNA sequence before being subsequently reacted by Click Chemistry with an azido-containing metal complex.[10,26] We have also recently extended this concept to an azido ligand, namely 2-azido-*N*,*N*-bis((pyridin-2-yl)methyl)ethanamine (**Dpa-N_3**, Figure 1), which was first coupled using Click Chemistry, on the solid phase, to an alkyne-containing PNA oligomer before being complexed with a ^{99m}Tc tricarbonyl derivative.[27]

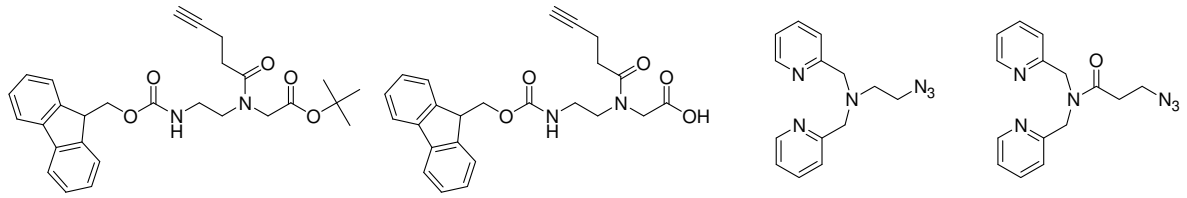


Figure 1. Structures of **Fmoc-1-O^{t}Bu**, **Fmoc-1-OH**, **Dpa-N$_3$** and **3**.

In order to increase the range of ligands, which can be reacted by Click Chemistry, we have recently embarked on a program to prepare new azido derivatives of the 2,2´-dipicolylamine (Dpa) ligand. It was anticipated that these compounds could also be reacted by Click Chemistry to alkyne-containing PNA oligomers before being coordinated to Re/^{99m}Tc carbonyl complexes. The examples of successful preparations of Re-containing PNA oligomers are surprisingly scarce,[23,26,28,29] whilst those of ^{99m}Tc-containing PNA oligomers are more common.[19,27,29-34] Re tricarbonyl PNA bioconjugates can be used as IR spectroscopical biosensors for DNA/RNA sequences. Indeed, the presence of intense carbonyl absorptions in the 1800-2200 cm^{-1} region render possible the selective detection and even quantification of the metal-PNA bioconjugates by IR spectroscopy (carbonyl metallo immuno assay, CMIA).[35-38] Our groups have also recently introduced metal carbonyl complexes as novel entities for RAMAN microscopy.[39] ^{99m}Tc-containing PNAs have also been prepared to be employed as bioimaging agents.[19,31,40-44]

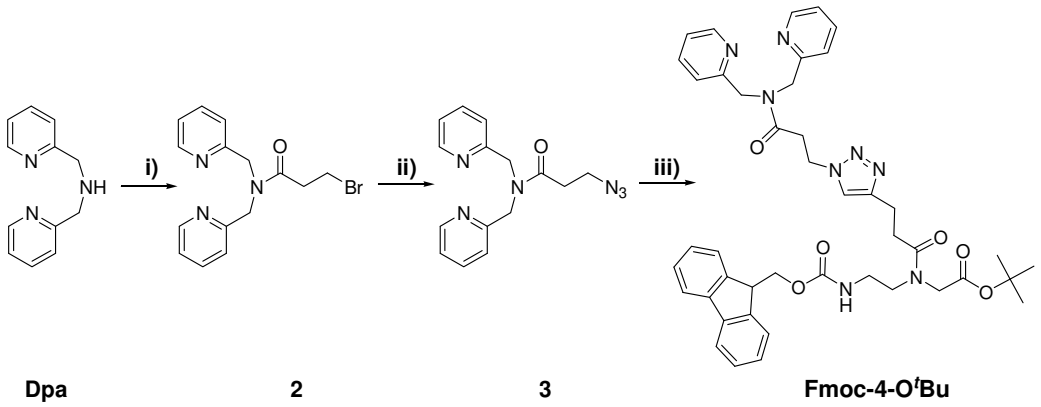
For the purpose of this work, we decided to use a di-(2-picolyl)amide (Dpam) derivative (**3**, Figure 1) as a coordination entity for Re/^{99m}Tc tricarbonyl complexes. Such compounds were shown to be excellent ligands for metal ions, especially for Cu(II), Cd(II) and Zn(II).[45-57] Noteworthy, Alsfasser, Vahrenkamp *et al.* have inserted such Dpam ligands to the side-chain of amino acids in order to introduce metal complexes in peptide frameworks for medicinal and biological applications.[45,47,48,52,53,57] However, to the best of our knowledge, no Re or Tc tricarbonyl complexes coordinated to such ligands have been reported to date.

Herein, we report on the successful preparation of a novel azido-Dpam derivative, namely 3-azido-*N,N*-bis-pyridin-2-ylmethyl-propionamide (**3**, Figure 1) and its subsequent couplings to the alkyne-containing PNA monomer **Fmoc-1-O^{t}Bu** and to a 17-mer alkyne-containing PNA oligomer (**Alkyne-PNA**) to give **4** and **Dpam-PNA**, respectively. Our attempts to complex **Dpam-PNA** with $[NEt_4]_2[ReBr_3(CO)_3]$ and the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ moiety are also discussed in detail.

Results and Discussion.

The synthetic route towards the preparation of **Fmoc-4-O^{t}Bu** is summarised in Scheme 1. In brief, 3-bromo-*N,N*-bis(pyridin-2-ylmethyl)propanamide (**2**) was synthesised by reacting the commercially available di-(2-picolyl)amine (Dpa) with 3-bromopropionyl chloride in dry $CHCl_3$. The presence of the expected compound was confirmed by NMR spectroscopy with the formation of two singlets at 4.74 and 4.77 ppm respectively in the ^{1}H-NMR spectrum of **2**, corresponding to the CO-N-CH_2-pyridine protons. These protons are no longer magnetically equivalent due to the formation of the amide bond. The bromo group of **2** was then replaced by an azido group using an excess of sodium azide in DMSO to give **3**. A sharp vibration band at 2102 cm^{-1} in the IR spectrum corresponding to the azide function validated the successful preparation of **3**. The Dpam-containing PNA monomer **Fmoc-4-O^{t}Bu** could then be prepared, in a relatively good yield (44%), by reacting **3** with the previously described alkyne-containing PNA monomer[10] (**Fmoc-1-O^{t}Bu**) in the presence of CuI and sodium ascorbate in a mixture of water and acetone. Instead of employing the usual combination of a Cu(II) salt and a reductant such as sodium ascorbate to form, *in situ*, the required Cu(I) species coordinating the alkyne function, CuI was used in this reaction. It was anticipated that this substitution could prevent the complexation of the Dpa ligand of **3** with Cu(II), which would have eventually avoided the formation of the desired **Fmoc-4-O^{t}Bu**. Moreover, to avoid the oxidation of Cu(I), sodium ascorbate was added to the reaction mixture and the reaction was carried out under an argon atmosphere. The presence of **Fmoc-4-O^{t}Bu** was ascertained by ESI-MS spectrometry with a peak at 773.29 *m/z* ascribed to $[M+H]^+$. Furthermore, as expected, the ^{1}H NMR spectrum of **Fmoc-4-O^{t}Bu**, which is quite complicated due to the presence of rotamers, no longer contains the alkyne proton peaks at 1.72 and 1.88 ppm respectively, which are present in the ^{1}H-NMR spectrum of **Fmoc-1-O^{t}Bu** (see Figures S1 and S2 in the SI for the ^{1}H- and ^{13}C-NMR spectra of **Fmoc-4-O^{t}Bu**).[10] However, a new signal at approximatively 7.40 ppm corresponding to the proton of the

triazole ring is observed in the ^{1}H-NMR spectrum of **Fmoc-4-O^{t}Bu**. These data together unambiguously confirmed the formation of **Fmoc-4-O^{t}Bu**.



Scheme 1. Preparation of **Fmoc-4-O^{t}Bu.** i) $Br(CH_2)_2COCl$, $CHCl_3$, 100%; ii) NaN_3, DMSO, 31%; iii) **Fmoc-1-O^{t}Bu**, CuI, sodium ascorbate, acetone:H_2O 2:1 V:V, 44%.

Due to the promising results obtained on the PNA monomer, we attempted to generalise this concept to a PNA oligomer. To do so, a 17-mer PNA oligomer containing the synthon **1** (**Alkyne-PNA**) was synthesised on a TentaGel R Fmoc-Lys(Boc)-RAM resin using Fmoc-Bhoc-protected PNA monomers (see Table 1 for a summary of the PNA oligomers prepared during the course of this work). The *C*-terminal lysine residue was introduced to enhance solubility[58] and the glycine residue was inserted at the *N*-terminus of the PNA sequence to avoid any *N*-acyl transfer reactions.[59] A spacer was also incorporated on each side of synthon **1** to avoid any steric hindrance. The resin containing **Alkyne-PNA** was then transferred into a fritted syringe and swollen with DMF for 1h. Using similar reaction conditions as those recently reported by our group,[27] the alkyne-containing PNA oligomer could be functionalised, on the solid phase, with **3** to give **Dpam-PNA**. The PNA oligomer was then cleaved from the resin with a mixture of TFA:TIS:H_2O 95:2.5:2.5 (V/V/V). After HPLC purification, both the success of the click chemistry reaction and the purity of the oligomer were determined by ESI-MS, MALDI-TOF and LC-MS (see experimental section for more

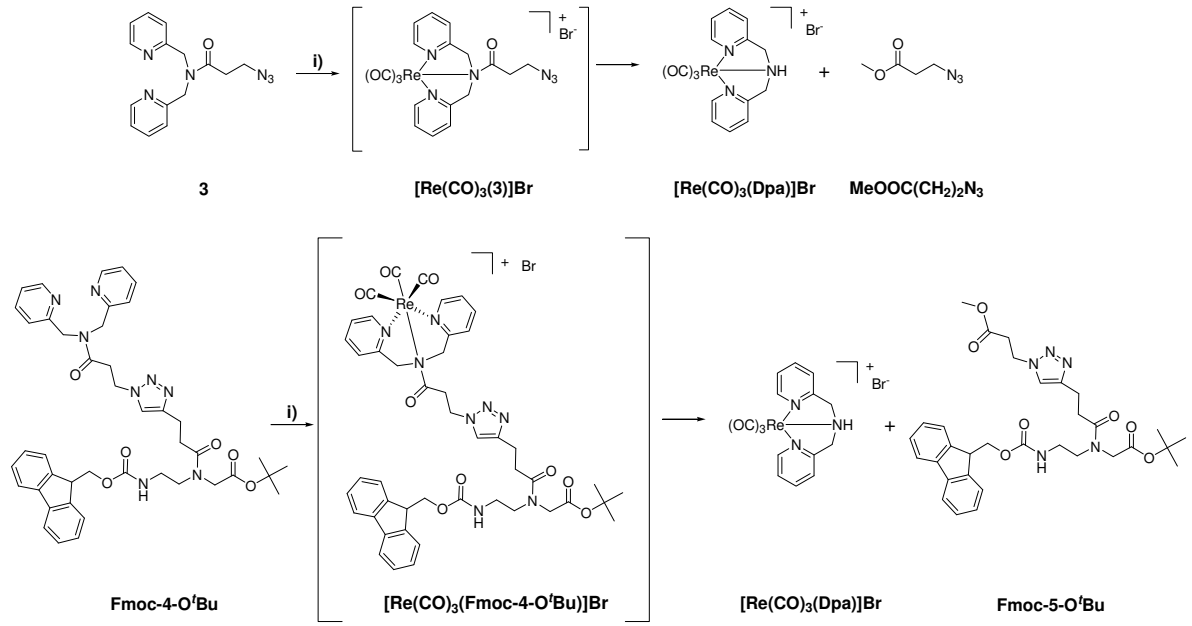
details). As expected, peaks at *m/z* 1422, 1138, 948, 813, 711, 632 and 569 ascribed to $[M+4H]^{4+}$, $[M+5H]^{5+}$, $[M+6H]^{6+}$, $[M+7H]^{7+}$, $[M+8H]^{8+}$, $[M+9H]^{9+}$ and $[M+10H]^{10+}$, respectively were observed in the ESI-MS spectrum (see Figure S3 in the SI for the ESI-MS spectrum of **Dpam-PNA**). Despite great care during the HPLC purification, a very small amount of a **Dpam-PNA** oligomer missing one G base (MM-291) and of a **Dpam-PNA** oligomer missing both one G base and one T base (MM-291-266) are evident in the ESI-MS spectrum of the "pure fraction" of **Dpam-PNA**. The presence of these two extra oligomers were confirmed by MALDI-TOF spectrometry with a major peak at *m/z* 5682.9 corresponding to $[M+H]^+$ and two minor peaks at *m/z* 5391.6 and 5127.3 corresponding to $[M+H-G]^+$ and $[M+H-G-T]^+$, respectively (see Figure S4 in the SI for the MALDI-TOF spectrum of **Dpam-PNA**). These observations are not very surprising as it is well-known that the purification of closely related PNA oligomers is extremely difficult. Nonetheless, the purity of **Dpam-PNA** is high enough to be used for the next synthetic step (see Figure S5 in the SI for the HPL chromatogram of **Dpam-PNA**).

Table 1. Summary of the PNA oligomers prepared in this work

Name	PNA Sequence
Alkyne-PNA	H-gly-gcggctgtgcggtgcgg-Spacer-**1**-Spacer-Lys-NH_2
Dpam-PNA	H-gly-gcggctgtgcggtgcgg-Spacer-**4**-Spacer-Lys-NH_2

With **Dpam-PNA** in hand, we have then focused our attention on examining the feasibility of complexing the Dpam moiety with $[NEt_4]_2[ReBr_3(CO)_3]$. However, as no Re tricarbonyl complexes coordinated to such ligands have been reported to date, we initially investigated the behaviour of **3** and **Fmoc-4-O^{t}Bu** upon metal complexation in methanol. Alfasser *et al.* have reported in detail the methanolysis of such ligands when reacted with Cu(II) salts,[45,47,53] however they did not observe such phenomena with Cd(II) salts.[53] Using standard

experimental conditions for the complexation of the Re tricarbonyl moiety with similar ligands,[26] namely refluxing in methanol for 3h using a 1:1 stoechiometry between the ligand and $[NEt_4]_2[ReBr_3(CO)_3]$, we discovered that a similar methanolysis of **3** occurred, as described in Scheme 2. ESI-MS analysis of the sticky yellow oil crude product confirmed the methanolysis with two intense peaks at *m/z* 469.9 and 130.1, corresponding to the expected degradation products **$[Re(CO)_3(Dpa)]^+$** [60] and **$[MeOOC(CH_2)_2N_3 + H]^+$** [61], respectively (see Figure S6 in the SI). A similar behaviour was observed when **Fmoc-4-O^{t}Bu** was refluxed in methanol with $[NEt_4]_2[ReBr_3(CO)_3]$ (Scheme 2). However, in this case, we were able to detect the presence of a small amount of the complex **$[Re(CO)_3$(Fmoc-4-O^{t}Bu)]Br** by MALDI-TOF spectrometry. The expected isotopic pattern for **$[Re(CO)_3$(Fmoc-4-O^{t}Bu)]$^+$** was found at approximatively *m/z* 1043 (see Figure S7 in the SI).



Scheme 2. Reaction of **3** and **Fmoc-4-O^{t}Bu** with $[NEt_4]_2[ReBr_3(CO)_3]$ in methanol engendering methanolysis. i) $[NEt_4]_2[ReBr_3(CO)_3]$, MeOH, reflux, 3h.

Due to these findings, we have decided to perform the labelling of **Dpam-PNA** with $[NEt_4]_2[ReBr_3(CO)_3]$ in acetonitrile instead of methanol. A similar stoichiometric ratio to that

successfully used for the labelling of a PNA oligomer with $[^{99m}Tc(CO)_3(H_2O)_3]^+$, namely an excess of the PNA oligomer compared to the metal ion (100:1), was employed.[27] However, after 1h at 80°C, no complexation (nor hydrolysis) of **Dpam-PNA** could be detected by MALDI-TOF spectrometry. Increasing the reaction time and the amount of the Re complex (up to a 1:1 ratio PNA:metal ion) as well as addition of water did not lead to any significant improvements. The only detectable peaks in the MALDI-TOF spectrum were those corresponding to the starting materials (see experimental section). However, when the labelling of **Dpam-PNA** with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ moiety was investigated, small amounts of ^{99m}Tc complexation could be detected, probably due to the higher sensitivity of the γ-detector. As shown in Figure 2, after a reaction time of 1h at 70°C, the most abundant ^{99m}Tc species present in solution (91 %) is the unreacted $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (t_R = 5.32 min). The three other peaks at t_R = 3.33, 17.20 and 18.97 min correspond to $[TcO_4]^-$ (2%), the expected ^{99m}Tc PNA oligomer **$[^{99m}Tc(CO)_3$(Dpam-PNA)$]^+$** (4%) and the ^{99m}Tc degradation product **$[^{99m}Tc(CO)_3$(Dpa)$]^+$** (3%), respectively. The assignment of the later peak could be ascertained by comparing the HPLC trace (γ detection) of the reaction of $[Tc(CO)_3(H_2O)_3]^+$ with **Dpa·3HCl** (see Figure S8 in the SI).[62] It should be noted that a definitive proof of the formation of **$[^{99m}Tc(CO)_3$(Dpam-PNA)$]^+$** cannot be given due to the absence of the cold Re analogue. However, the retention time (t_R =17.20) corroborates with that previously described for a Dpa-containing PNA oligomer.[27] Indeed, **$[^{99m}Tc(CO)_3$(Dpa)$]^+$** was found to be more lipophilic than the bioconjugates itself **$[^{99m}Tc(CO)_3$(Dpam-PNA)$]^+$.** Furthermore, upon *fac*-$[Tc(CO)_3]^+$ complexation of **Dpam-PNA**, **$[^{99m}Tc(CO)_3$(Dpam-PNA)$]^+$** also became more hydrophilic (shift from 20.75 to 17.20 min in the HPL chromatograms). Noteworthy, the unreacted PNA oligomer **Dpam-PNA** could still be detected by HPLC (UV detection) after 1h at 70°C, emphasising the stability of PNAs even under harsh conditions (see Figure S9 in the SI). The presence of **$[^{99m}Tc(CO)_3$(Dpa)$]^+$** underscores that hydrolysis is difficult to avoid. It is worth mentioning that Alberto *et al.* have also encountered a C-N bond cleavage of one

of their ligands upon ^{99m}Tc complexation.[63] All in all, the complexation yields of **Dpam-PNA** with both $[NEt_4]_2[ReBr_3(CO)_3]$ and the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ moiety are either non-existent or extremely poor. These findings suggest that **Dpam** is not an appropriate ligand for both IR biosensing or radiolabelling purposes, at least under the experimental conditions employed in this study.

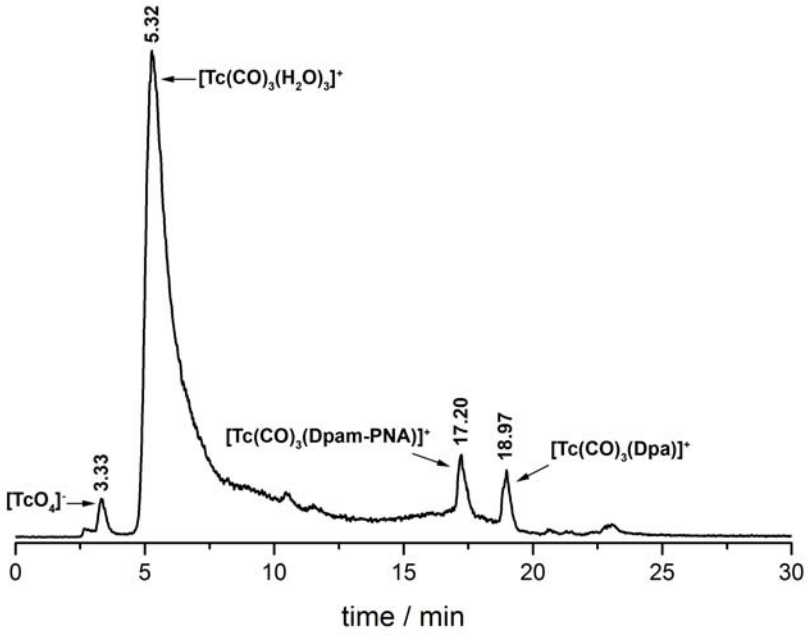


Figure 2. HPLC trace (γ detection) of the reaction of $[Tc(CO)_3(H_2O)_3]^+$ with **Dpam-PNA** after 60 min at 70°C.

Conclusions

In this study, we have demonstrated, to the best of our knowledge, the first successful insertion of a modified di-(2-picolyl)amide (Dpam) ligand into both a PNA monomer and a 17-mer PNA oligomer using click chemistry methodology. Furthermore, it was shown that two Dpam-containing derivatives, **3** and **Fmoc-4-O^{t}Bu**, behave similarly upon complexation with Re(I) or with Cu(II) with regards of methanolysis. Indeed, upon reaction of the two latter compounds with $[NEt_4]_2[ReBr_3(CO)_3]$ in methanol, the amide bond of the two expected Re(I) complexes are cleaved, as previously observed by Alsfasser and co-workers upon complexation of similar ligands with Cu(II). However, no complexation or hydrolysis of **Dpam-PNA** could be detected by MALDI-TOF spectrometry when Dpam-PNA was reacted with $[NEt_4]_2[ReBr_3(CO)_3]$ for 1h at 80°C in acetonitrile. On the contrary, when the labelling of **Dpam-PNA** with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ moiety was examined, the expected ^{99m}Tc PNA oligomer **$[^{99m}Tc(CO)_3$(Dpam-PNA)$]^+$** and the ^{99m}Tc degradation product **$[^{99m}Tc(CO)_3$(Dpa)$]^+$** could be detected, although the most abundant ^{99m}Tc species present in solution was by far the unreacted $[^{99m}Tc(CO)_3(H_2O)_3]^+$. The extremely low yields obtained for both the Re and ^{99m}Tc complexation suggest that **Dpam** is not a suitable ligand for both IR biosensing or radiolabelling purposes under the experimental conditions presented in this study.

In a more general term, this work is another contribution towards the preparation of novel metal-containing PNA oligomers, which have shown great promises in various fields of research ranging from biosensing to nuclear therapy or diagnostics.[19] Moreover, the recent discovery of Re tricarbonyl complexes as potential anticancer agents is further encouragements to prepare new Re-PNA oligomers, where the PNAs could be used as specific transporters to deliver metallo-chemotherapeutics.[64] Alternatively, the Re complexes

could serve as fluorescent probes and not as anticancer agents, as recently demonstrated in a biological context.[23,26,65,66]

Experimental Section

Materials. All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over 4 Å molecular sieves.

Instrumentation and methods. ^{1}H- and ^{13}C-NMR spectra were recorded in deuterated solvents on a Bruker DRX 400 spectrometer at 30°C. The chemical shifts, δ, are reported in ppm (parts per million). The residual solvent peaks have been used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and br (broad). Infrared spectra were recorded on a ATR unit using a Bruker Tensor 27 FTIR spectrophotometer at 4 cm^{-1} resolution. Signal intensity is abbreviated br (broad). ESI mass spectra were recorded on a Bruker Esquire 6000. The matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) mass spectra were measured on a Bruker Daltonics Autoflex. The experiments were performed in linear mode with positive polarity using sinapinic acid or α-cyano-4-hydroxy-cinnamic acid as the matrix. *HPLC purification* of **Dpam-PNA** was performed on a Merck-Hitachi L-7000 system equipped with a diode array UV/Vis spectrometer and an Agilent Zorbax 300SB-C18 semi-prep column (5 μm particle size, 300 Å pore size, 250 x 9.4 mm. Flow rate: 4 ml min^{-1}). The runs were performed with a linear gradient of A (distilled water containing 0.1 % v/v TFA) and B (acetonitrile (Sigma-Aldrich HPLC-grade), containing 0.1 % v/v TFA). Preparative runs: t = 0 min 5 % B. t = 12 min 15 % B. t = 32 min 40 % B. t = 50 min 80 % B. t = 51 min 100 % B. t = 56 min 100 % B. t = 61 min 5 % B. *LC-MS spectrum* of **Dpam-PNA** was measured on a Acquity™ from Waters system equipped with a PDA detector and an auto sampler using an Agilent Zorbax 300SB-C18 analytical column (3.5 μm particle size, 300 Å pore size, 150 x 4.6 mm). This LC was coupled to an Esquire HCT from Bruker (Bremen, Germany) for the MS measurements. The LC run (flowrate: 0.3 mL min^{-1}) was performed with a linear gradient of A (distilled water

containing 0.1 % v/v formic acid) and B (acetonitrile (Sigma-Aldrich HPLC-grade), containing 0.1 % v/v formic acid); t = 0 min 5 % B. t = 3 min 5 % B. t = 17 min 100 % B. t = 20 min 100 % B. t = 25 min 5 % B.

Synthesis and Characterisation.

$[NEt_4]_2[ReBr_3(CO)_3]$. $[NEt_4]_2[ReBr_3(CO)_3]$ was prepared following the procedure published by Alberto *et al*. The spectroscopic data of the products matched that reported previously.[67]

Dpa·3HCl. Dpa·3HCl was prepared following the procedure published by Larsen *et al*.[68] The spectroscopic data of the products matched that reported previously.[68]

{[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-ethyl]-pent-4-ynoyl-amino}-acetic acid *tert*-butyl ester (Fmoc-1-O^{t}Bu). Fmoc-1-O^{t}Bu was prepared following the procedure published by Gasser *et al*.[10] The analytical data match what previously reported.[10]

2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetic acid (Fmoc-1-OH). Fmoc-1-OH was prepared following the procedure published by Gasser *et al*.[10] The analytical data match what previously reported.[10]

3-Bromo-*N*,*N*-bis(pyridin-2-ylmethyl)propanamide (2). To a stirred solution of di-(2-picolyl)amine (2.50 g, 12.10 mmol) in dry $CHCl_3$ (125 mL) cooled to 0°C was added 3-bromopropionyl chloride (2.18 g, 12.10 mmol) under a nitrogen atmosphere. The reaction mixture was stirred for 30 minutes at 0°C and then for 16h at room temperature. The reaction mixture turned light green and then yellow. An aqueous solution of $Na_2CO_{3(sat)}$ (100 mL) was then added to this mixture which was then stirred for 5 min. The phases were separated and the aqueous phase was back-extracted with $CHCl_3$ (2x150 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvent removed under vacuo to give a brown-orange oil that is pure enough to be used for the next synthetic reaction step. Yield: 4.04g (100 %). **Characterisation Data.** ^{1}H-NMR Spectrum ($CDCl_3$): δ 3.07 (t, ^3J = 7.0 Hz, 2H, NCO-CH_2-CH_2), 3.67 (t, ^3J = 7.0 Hz, 2H, CH_2-CH_2Br), 4.74 (s, 2H, CO-N-CH_2-pyr), 4.77 (s, 2H, CO-N-

C*H*$_2$-pyr), 7.14 (m, 2H, C*H* pyr), 7.29 (m, 2H, C*H* pyr), 7.61 (m, 2H, C*H* pyr), 8.50 (m, 2H, C*H* pyr).^{13}C-NMR Spectrum ($CDCl_3$): δ 27.37 (CH_2-*C*H_2-Br), 36.41 (CO-CH_2-*C*H_2Br), 51.37 (N-*C*H_2-pyr), 52.83 (N-*C*H_2-pyr), 121.05 (*C*H pyr), 122.28 (*C*H pyr), 122.50 (*C*H pyr), 122.54 (*C*H pyr), 136.69 (*C*H pyr), 136.75 (*C*H pyr), 149.04 (*C*H pyr), 149.87 (*C*H pyr), 156.07 (*C* pyr), 156.99 (*C* pyr), 171.03 (N*C*O). Electrospray Mass Spectrum (m/z): 254.1 [M-Br]$^+$ (100%).

3-Azido-*N*,*N*-bis(pyridin-2-ylmethyl)propanamide (3). Sodium azide (0.68 g, 10.45 mmol) was partially dissolved while stirring in dry DMSO (20 mL) for 1h30 under a nitrogen atmosphere. **2** (0.70 g, 2.09 mmol) diluted in dry DMSO (5 mL) was added to the solution and, within minutes, the remaining sodium azide dissolved. The reaction was stirred for 22h at room temperature. The reaction mixture was then diluted with H_2O (45 mL) and the product extracted with ethyl acetate (2 x 75 mL). The combined organic phases were washed with brine (3 x 40 mL), dried with Na_2SO_4, filtered and concentrated under vacuum to give a brown oil. The product was purified by column chromatography on silica with ethyl acetate:methanol 10:1 as the eluent to give **3** as a slightly yellow oil. It is to point out that two spots are observed by TLC (Rfs = 0.28 and 0.32) while the product looks nearly perfectly pure by both ^{1}H- and ^{13}C-NMR spectroscopy. Yield: 0.19 g (31 %). **Characterisation Data.** Major IR bands (ν, cm^{-1}): 3051 w, 3009 w, 2934 w, 2102 m, 1733 m, 1645 s, 1591 m, 1570 m, 1474 m, 1434 s, 1371 w, 1359 w, 1290 m, 1268 m, 1240 m, 1198 m, 1148 w, 1098 w, 1047 m, 994 m, 969 w, 937 w, 902 w, 839 w, 751 s, 634 m, 617 m. ^{1}H-NMR Spectrum ($CDCl_3$): δ 2.76 (t, ^3J = 6.5 Hz, 2H, NCO-C*H*$_2$-CH_2), 3.66 (t, ^3J = 6.5 Hz, 2H, CH_2-C*H*$_2$Br), 4.70 (s, 2H, CO-N-C*H*$_2$-pyr), 4.77 (s, 2H, CO-N-C*H*$_2$-pyr), 7.15 (m, 2H, C*H* pyr), 7.29 (m, 2H, C*H* pyr), 7.62 (m, 2H, C*H* pyr), 8.51 (m, 2H, C*H* pyr). ^{13}C-NMR Spectrum ($CDCl_3$): δ 32.65 (NCO-*C*H_2-CH_2), 47.28 (CH_2-*C*H_2N3), 51.46 (N-*C*H_2-pyr), 52.94 (N-*C*H_2-pyr), 121.02 (*C*H pyr), 122.32 (*C*H pyr), 122.57(*C*H pyr), 122.60 (*C*H pyr), 136.69 (*C*H pyr), 136.80 (*C*H pyr), 149.14 (*C*H pyr), 149.95 (*C*H pyr), 156.22 (*C* pyr), 157.12 (*C* pyr), 171.04 (N*C*O).

Electrospray Mass Spectrum (m/z): 254.1 $[M\text{-}N_3]^+$ (10%), 297.1 $[M+H]^+$ (100%), 319.1 $[M+Na]^+$ (10%).

{(3-{1-[2-(Bis-pyridin-2-ylmethyl-carbamoyl)-ethyl]-1H-[1,2,3]triazol-4-yl}-propionyl)-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino}-acetic acid tert-butyl ester (Fmoc-4-O^{t}Bu). To a suspension of **3** (70 mg, 0.236 mmol) and **Fmoc-1-O^{t}Bu** (100 mg, 0.236 mmol) in a mixture of acetone (3 mL) and water (1.5 mL), CuI (9 mg, 47.2 µmol) and sodium ascorbate (19 mg, 94.0 µmol) were successively added. The reaction mixture was stirred for 16h under an Ar atmosphere at room temperature. After evaporation of the solvents, dichloromethane (40 mL) was added and the solution was then washed twice with water (2x10 mL). The organic yellow phase was then dried over Na_2SO_4, filtered and evaporated to dryness. The orange residue was purified by chromatography column on silica with ethyl acetate:methanol (10:1) as the eluent to give **Fmoc-4-O^{t}Bu** as a slightly yellow oil. Yield: 80 mg (44%). **Characterisation Data.** ^{1}H-NMR spectrum ($CDCl_3$) δ: 1.42 (s, 9H, *tert*-Butyl), 2.55 (m, 2H, $C_{triazole}$-CH_2-C*H*$_2$-CO), 2.95 (m, 2H, $C_{triazole}$-C*H*$_2$-CH_2-CON), 2.99 - 3.10 (m, NCO-C*H*$_2$-CH_2-$N_{triazole}$), 3.27 (m, 2H, OCONH-C*H*$_2$-CH_2-N), 3.42 (m, 2H, OCONH-CH_2-C*H*$_2$-N), 3.85 and 3.90 (d, rotamers, 2H, N-C*H*$_2$-$COOC(CH_3)_3$), 4.05 - 4.17 (m, 1H, C_{Fmoc}-C*H*-CH_2-OCON), 4.27 (m, 2H, C_{Fmoc}-CH-C*H*$_2$-OCONH), 4.52 (d, rotamers, 4H, $C_{pyridine}$-C*H*$_2$-N), 4.60 (m, NCO-CH_2-C*H*$_2$-$N_{triazole}$), 6.95 (t, ^{3}J = 8.00 Hz, 2H, C*H* pyridine), 7.05 - 7.12 (m, 2H, C*H* pyridine), 7.19 - 7.25 (m, 2H, C*H* Fmoc), 7.27 - 7.35 (m, 2H, C*H* Fmoc), 7.40 (s, 1H, C*H* triazole), 7.49 - 7.58 (m, 4H, C*H* Fmoc and C*H* pyridine), 7.69 (d, ^{3}J = 4.67 Hz, 2H, C*H* Fmoc), 8.40 (m, 2H, C*H* pyridine). ^{13}C-NMR spectrum ($CDCl_3$) δ: 21.10 ($C_{triazole}$-*C*H_2-CH_2-CON), 28.04 (*tert*-Butyl *C*H_3), 31.90 ($C_{triazole}$-CH_2-*C*H_2-CON), 33.62 (NCO-*C*H_2-CH_2-$N_{triazole}$), 47.76 (C_{Fmoc}-*C*H-CH_2-OCON), 48.20 (OCONH-CH_2-*C*H_2-N), 49.89 (OCONH-*C*H_2-CH_2-N), 49.95 (NCO-CH_2-*C*H_2-$N_{triazole}$), 51.43 ($C_{pyridine}$-C*H*$_2$-N), 52.79 (N-*C*H_2-$COOC(CH_3)_3$), 67.00 (C_{Fmoc}-CH-C*H*$_2$-OCONH), 82.94 (N-CH_2-$COOC(CH_3)_3$), 119.90 (*C*H Fmoc), 121.21 (*C*H pyridine), 122.46 (*C*H triazole), 122.63 (*C*H pyridine),

125.14 (*C*H Fmoc), 127.06 (*C*H Fmoc), 127.67 (*C*H Fmoc), 136.70 (*C*H pyridine), 141.27 (*C* Fmoc), 143.91 (*C* Fmoc), 146.31 (*C* triazole), 149.94 (*C*H pyridine), 155.80 (O*C*ONH), 156.96 (*C* pyridine), 169.00 and 170.00 (rotamers, N-CH_2-*C*OOC$(CH_3)_3$), 170.64 (N*C*O-CH_2-CH_2-$N_{triazole}$), 172.53 and 173.35 (rotamers, $C_{triazole}$-CH_2-CH_2-*C*ON), ESI-MS [m/z]: 773.29 $[M+H]^+$.

Attempt of preparation of the $Re(CO)_3$ complex of 3 ([$Re(CO)_3$(3)]Br). $[NEt_4]_2[ReBr_3(CO)_3]$ (0.259 g, 0.34 mmol) and **3** (0.100 g, 0.34 mmol) were refluxed in deoxygenated methanol (40 mL) under an Ar atmosphere for 3 hours. The mixture was then cooled to room temperature and evaporated to dryness to give a sticky yellow oil. ESI-MS analysis of the crude product confirmed the methanolysis with two intense peaks at m/z 469.9 and 130.1 corresponding to the expected compounds **[$Re(CO)_3$(Dpa)]$^+$** and [**$MeOOC(CH_2)_2N_3$** + H]$^+$, respectively (see Figure S5 in the SI). These compounds were not isolated.

Attempt of preparation of the $Re(CO)_3$ complex of Fmoc-4-O^{t}Bu ([$Re(CO)_3$(Fmoc-4-O^{t}Bu)]Br). To a stirring solution of $[NEt_4]_2[Re(CO)_3Br_3]$ (79.33 mg, 0.104 mmol) in deoxygenated methanol (15 mL), **Fmoc-4-O^{t}Bu** (80 mg, 0.104 mmol), dissolved in deoxygenated methanol (1.5 mL), was added under an Ar atmosphere. The reaction mixture was refluxed for 3 hours. The mixture was then cooled to room temperature and evaporated to dryness to give a yellow oil. ESI-MS analysis of the crude product confirmed the methanolysis with two intense peaks at *m/z* 470.0 and 628.2 corresponding to the expected compounds of methanolysis **[$Re(CO)_3$(Dpa)]$^+$** and **Fmoc-5-O^{t}Bu**, respectively. These compounds were not isolated. However, a small amount of the expected compound **[$Re(CO)_3$(Fmoc-4-O^{t}Bu)]Br** could be detected by MALDI-TOF with peaks at *m/z* 1041.32 (53%), 1042.32 (36%), 1043.32 (100%), 1044.32 (55%) and 1045.33 (26 %) corresponding to $[M^+]$ (see Figure S7 in the SI for the isotopic pattern).

Synthesis of Alkyne-PNA. Alkyne-PNA was synthesised using an automated Expedite 8909 nucleic acid synthesiser (Applied Biosystems) adapted for PNA synthesis. Synthesis was performed on a 2-μmol scale with Tentagel R Ram-Lys(Boc)Fmoc resin (0.20 mmol/g) from Rapp Polymer using Fmoc/Bhoc-protected monomers from commercial suppliers (Link Technologies, Lanarkshire, Scotland). Synthon **Fmoc-1-OH**, dissolved in *N*-methylpyrrolidone (NMP), was inserted in one of the free positions of the synthesiser and a double coupling was applied in order to ensure a full coupling.

Synthesis of Dpam-PNA. The resin containing **Alkyne-PNA** was transferred into a fritted syringe. The resin was then swollen with DMF for 1h. **3** (1.7 mg, 6 μmol) and CuI (0.78 mg, 2 μmol) were then introduced into the syringe (from the top). Afterwards, a mixture of ethyldiisopropylamine (54 μL) and DMF (400 μl) were aspired up the syringe and the mixture was shaken for 2 days at room temperature in the absence of light and under an argon atmosphere. The resin was then washed with DMF (5x), CH_3CN (5x), CH_2Cl_2 (5x) and DMF (5x) successively. Before cleavage, the resin was shrunk with methanol and dried under high vaccum. The PNA oligomer was then cleaved using a mixture of trifluoroacetic acid:water:triisopropylsilane 95:2.5:2.5 [3 x 400 μl (1h30 each)]. The resulting solution was first evaporated to dryness before being precipitated with ice-cold ether. The solid was centrifuged, washed with ice-cold ether and finally air dried. The obtained crude oligomer was then purified by RP-HPLC and finally characterised by ESI-MS spectrometry. The purity of **Dpam-PNA** was checked by LC-MS. Despite great care taken during the HPLC purification, a very small amount of a **Dpam-PNA** oligomer missing one G base (MM-291) and of a **Dpam-PNA** oligomer missing both one G base and one C base (MM-291-266) are noticeable in the ESI-MS spectrum of **Dpam-PNA**. **Characterisation of Dpam-PNA.** HPLC: t_R = 10.2 min. ESI-MS: *m/z* 1422 $[M+4H]^{4+}$, 1138 $[M+5H]^{5+}$, 948 $[M+6H]^{6+}$, 813 $[M+7H]^{7+}$, 711 $[M+8H]^{8+}$, 632 $[M+9H]^{9+}$ and 569 $[M+10H]^{10+}$. MALDI-TOF: *m/z* 5682.9 $[M+H]^{+}$, 5391.6 $[M+H-G]^{+}$, 5127.3 $[M+H-G-T]^{+}$.

PNA concentration determination. The PNA strand concentration was measured on a NanoDrop 2000 spectrophotomer by means of the absorption at 260 nm using the incremental extinction coefficients of the PNA nucleobases ($\varepsilon_{PNA,A}$ = 13700 $M^{-1}cm^{-1}$, $\varepsilon_{PNA,G}$ = 11700 $M^{-1}cm^{-1}$, $\varepsilon_{PNA,C}$ = 6600 $M^{-1}cm^{-1}$, $\varepsilon_{PNA,T}$ = 8600 $M^{-1}cm^{-1}$).[2] An extinction coefficient of ε_{260} = 6099 $M^{-1}cm^{-1}$ was taken for the **Dpam** moiety. This value was determined for a similar **Dpa** derivative.[27]

Attempt of preparation of the $Re(CO)_3$ complex of Dpam-PNA ([$Re(CO)_3$(Dpam-PNA]Br). $[NEt_4]_2[Re(CO)_3Br_3]$ ($1.85 \cdot 10^{-10}$ g, 0.24 μmol) and **Dpam-PNA** (0.14 μg, 24.0 μmol) were reacted in acetonitrile (60 μl) for 1 h at 80 °C. The reaction mixture was then analysed by MALDI-TOF mass spectrometry. **Characterisation of the reaction mixture.** MALDI-TOF: m/z 5683.1 [M(**Dpam-PNA**)+H]$^+$, 5391.1 [M(**Dpam-PNA**)+H-G]$^+$, 5125.4 [M(**Dpam-PNA**)+H-G-T]$^+$, 5910.4 [M(**Dpam-PNA**)+SA]$^+$, 6131.9 [M(**Dpam-PNA**)+2SA]$^+$.

Radiolabeling experiments of Dpa and Dpam-PNA with ^{99m}Tc. Na[$^{99m}TcO_4$] was eluted from a ^{99}Mo/^{99m}Tc generator, using 0.9% saline (107 MBq). With an Isolink kit (Mallinckrodt-Tyco, Inc.), the preparation of *fac*-[$^{99m}Tc(CO)_3(H_2O)_3]^+$ was performed according to a previously described procedure.[69] The [$^{99m}Tc(CO)_3(H_2O)_3]^+$ solution was neutralised by the addition of 70 μL of a 1M HCl solution. To label the **Dpa** chelator with ^{99m}Tc, 100 μL of a 10^{-2} M Dpa·3HCl PBS solution were added to 100 μL of the [$^{99m}Tc(CO)_3(H_2O)_3]^+$ solution. The reaction was performed in a 1 ml Eppendorf tube at 95°C. After 20 min, the reaction was checked by HPLC.

For the radiolabeling of **Dpam-PNA**, 70 μL ($4.27 \cdot 10^{-5}$ μmol) of **Dpam-PNA** dissolved in CH_3CN were added to a 100 μL **[$^{99m}Tc(H_2O)_3(CO)_3]^+$** solution. The reaction was performed in a nitrogen-purged 1 mL Eppendorf vial. The solution was heated for 60 minutes at 70°C. Afterwards, the reaction was checked by HPLC.

HPLC analyses were performed on a Merck Hitachi LaChrom L 7100 pump coupled to a Merck Hitachi LaChrom L7200 tunable UV detector and a radiodetector, separated by a

Teflon tube, which causes about a 0.4 – 0.7 min delay compared to UV/vis detection. UV/vis detection was performed at 250 nm. The detection of radioactive ^{99m}Tc complexes was performed with a Berthold FlowStar LB513 radiodetector equipped with a NaI(Tl) scintillation detector. Separations were achieved on a Macherey-Nagel C18 reversed-phase column (Nucleosil 100 Å, 250 x 3 mm). The column was eluted with a flow rate of 0.5 ml min^{-1} using as eluents 0.1% TFA in H_2O (solvent A) and methanol (solvent B) with a variable gradient (0–3 min, 100% A; 3 – 3.1 min, 0 to 25% B; 3.1–9 min, 25% B; 9–9.1 min, 25% B to 34% B; 9.1–20 min, 34% B to 100% B; 20–25 min, 100% B; 25–25.1 min 100% B to 100% A; 25.1–30 min 100% A).

Acknowledgments.

This work was supported by the Swiss National Science Foundation (Ambizione Fellowship N° PZ00P2_126404 and Research Project N° 200021_129910 to G.G. as well as an Ambizione Fellowship N° PZ00P2_126414 to H.B.), the Alexander von Humboldt Foundation (fellowship to G.G.), the Research Department Interfacial Systems Chemistry of the Ruhr-University Bochum, and the DFG through the Research Unit "Biological Function of Organometallic Compounds" (FOR 630, www.rub.de/for630). The authors are grateful to *Dr. Jacqui F. Young* for her kind help with the MALDI-TOF measurements. G.G and H.B. thank *Prof. Roger Alberto* for generous access to all the facilities of the Institute of Inorganic Chemistry of the University of Zurich.

Supporting Information available. ^{1}H NMR and ^{13}C NMR spectra of **Fmoc-4-O^{t}Bu** (Figures S1-S2), ESI-MS and MALDI-TOF spectra of **Dpam-PNA** (Figures S3-S4), HPLC chromatogram of **Dpam-PNA** (Figure S5), ESI-MS of the methanolysis of **[Re(CO)$_3$(3)]Br** (Figure S6), MALDI-TOF spectrum of **[Re(CO)$_3$(Fmoc-4-O^{t}Bu)]Br** (Figure S7), HPLC trace (γ detection) of the reaction of $[Tc(CO)_3(H_2O)_3]^+$ with **Dpa·3HCl** (Figure S8) and HPLC trace (UV detection) of the reaction of $[Tc(CO)_3(H_2O)_3]^+$ with **Dpam-PNA** (Figure S9).

References

(1) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchhardt, O. *Science* **1991**, *254*, 1497-1500.

(2) *Peptide Nucleic Acids, Protocols and Applications*; Nielsen, P. E.; Egholm, M., Eds.; Horizon Scientific Press: Wymondham, UK, 1999.

(3) Egholm, M.; Buchhardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566-568.

(4) Jensen, K. K.; Orum, H.; Nielsen, P. E.; Norden, B. *Biochemistry* **1997**, *36*, 5072-5077.

(5) Demidov, V. V.; Potaman, V. N.; Frank-Kamenetskii, M. D.; Egholm, M.; Buchhardt, O.; Sönnichsen, S. H.; Nielsen, P. E. *Biochem. Pharmacol.* **1994**, *48*, 1310.

(6) Koppelhus, U.; Nielsen, P. E. In *Antisense Drug Technology*; Crooke, S. T., Ed.; Marcel Dekker: New York, 2001, p 359-374.

(7) Lundin, K. E.; Good, L.; Strömberg, R.; Gräslund, A.; Smith, C. I. E. *Adv. Genetics* **2006**, *56*, 1-51, and references therein.

(8) Nielsen, P. E. *ChemBioChem* **2010**, *11*, 2073–2076, and references therein.

(9) Wang, J. In *Peptide Nucleic Acids, Protocols and Applications*; Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific Press: Wymondham, Uk, 1999, p 155-161, and references therein.

(10) Gasser, G.; Hüsken, N.; Köster, S. D.; Metzler-Nolte, N. *Chem. Comm.* **2008**, 3675-3677.

(11) Gasser, G.; Belousoff, M. J.; Bond, A. M.; Spiccia, L. *J. Org. Chem.* **2006**, *71*, 7565-7573.

(12) Gasser, G.; Spiccia, L. *J. Organomet. Chem.* **2008**, *693*, 2478-2482.

(13) Gasser, G.; Neukamm, M. A.; Ewers, A.; Brosch, O.; Weyhermüller, T.; Metzler-Nolte, N. *Inorg. Chem.* **2009**, *48*, 3157-3166.

(14) Sosniak, A.; Gasser, G.; Metzler-Nolte, N. *Org. Biomol. Chem.* **2009**, *7*, 4992 – 5000.

(15) Patra, M.; Gasser, G.; Bobukhov, D.; Merz, K.; Shtemenko, A. V.; Metzler-Nolte, N. *Dalton Trans.* **2010**, *39*, 5617-5619.

(16) Nickita, N.; Gasser, G.; Bond, A. M.; Spiccia, L. *Eur. J. Inorg. Chem.* **2009**, *14*, 2179-2186.

(17) Hüsken, N.; Gasser, G.; Köster, S. D.; Metzler-Nolte, N. *Bioconjugate Chem.* **2009**, *20*, 1578-1586.

(18) Gasser, G.; Brosch, O.; Ewers, A.; Weyhermüller, T.; Metzler-Nolte, N. *Dalton Trans.* **2009**, 4310-4317.

(19) Gasser, G.; Sosniak, A. M.; Metzler-Nolte, N. **2010**, submitted, and references therein.

(20) Metzler-Nolte, N. In *Bioorganometallics: Biomolecules, Labeling, Medicine*; Jaouen, G., Ed.; Wiley-VCH: Weinheim, 2006.

(21) Metzler-Nolte, N.; Salmain, M. In *Ferrocenes: Ligands, Materials and Biomolecules*; Stepnicka, P., Ed.; John Wiley & Sons Ltd.: Chichester, UK 2008, p 499-639.

(22) Metzler-Nolte, N.; Severin, K. In *Concepts and Models in Bioinorganic Chemistry*; Kraatz, H.-B., Metzler-Nolte, N., Eds.; Wiley-VCH Verlag GmbH & Co: Weinheim, Germany, 2006, p 113-136.

(23) Ferri, E.; Donghi, D.; Panigati, M.; Prencipe, G.; D'Alfonso, L.; Zanoni, I.; Baldoli, C.; Maiorana, S.; D'Alfonso, G.; Licandro, E. *Chem. Comm.* **2010**, *46*, 6255-6257

(24) Tørnoe, C. W.; Christensen, C.; Meldal, L. *J. Org. Chem.* **2002**, *67*, 3057-3064.

(25) Rostovtsev, V. V.; Green, M. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596-2599.

(26) Gasser, G.; Pinto, A.; Neumann, S.; Sosniak, A. M.; Seitz, M.; Merz, K.; Heumann, R.; Metzler-Nolte, N. **2010**, submitted.

(27) Gasser, G.; Jäger, K.; Zenker, M.; Bergmann, R.; Steinbach, J.; Stephan, H.; Metzler-Nolte, N. *J. Inorg. Biochem.* **2010**, *104*, 1133-1140.

(28) Hamzavi, R.; Happ, T.; Weitershaus, K.; Metzler-Nolte, N. *J. Organomet. Chem.* **2004**, *689*, 4745-4750.

(29) Xavier, C.; Giannini, C.; Gano, L.; Maiorana, S.; Alberto, R.; Santos, I. *J. Biol. Inorg. Chem.* **2008**, *13*, 1335-1344.

(30) Tian, X.; Aruva, M. R.; Qin, W.; Zhu, W.; Duffy, K. T.; Sauter, E. R.; Thakur, M. L.; Wickstrom, E. *J. Nucl. Med.* **2004**, *45*, 2070-2082.

(31) Tian, X.; Mohan, R.; Qin, W.; Zhu, W.; Sauter, E. R.; Thakur, M. L.; Wickstrom, E. *Bioconjugate Chem.* **2005**, *16*, 70−76.

(32) Rao, P. S.; Tian, X.; Qin, W.; Aruva, M. R.; Thakur, M. L.; Wickstrom, E. *Nucl. Med. Commun.* **2003**, *24*, 857-863.

(33) Mardirossian, G.; Lei, K.; Rusckowski, M.; Chang, F.; Qu, T.; Egholm, M.; Hnatowich, D. J. *J. Nucl. Med.* **1997**, *38*, 907-913.

(34) Mier, W.; Eritja, R.; Mohammed, A.; Haberkorn, U.; Eisenhut, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 1968-1971.

(35) Salmain, M.; Vessières, A.; Brossier, P.; Butler, I. S.; Jaouen, G. *J. Immunol. Meth.* **1992**, *148*, 65-75.

(36) Salmain, M.; Vessieres, a.; Varenne, A.; Brossier, P.; Jaouen, G. *J. Organomet. Chem.* **1999**, *589*, 92-97.

(37) Vessieres, A.; Salmain, M.; Brossier, P.; Jaouen, G. *J. Pharm. Biomed. Anal.* **1999**, *21*, 625-633.

(38) Fischer-Durand, N.; Salmain, M.; Rudolf, B.; Vessieres, A.; Zakrzewski, J.; Jaouen, G. *ChemBioChem* **2004**, *5*, 519-525.

(39) Meister, K.; Niesel, J.; Schatzschneider, U.; Metzler-Nolte, N.; Schmidt, D.; Havenith, M. **2009**, submitted.

(40) Wickstrom, E.; Tian, X.; Amirkhanov, N. V.; Chakrabarti, A.; Aruva, M. R.; Rao, P. S.; Qin, W.; Zhu, W.; Sauter, E. R.; Thakur, M. L. In *Antisense Therapeutics*; 2nd ed.; Phillips, M. I., Ed.; Humana Press: Totowa, 2005; Vol. 106, p 135-191.

(41) Wickstrom, E.; Thakur, M. L.; Sauter, E. R. In *Peptide Nucleic Acids, Morpholinos, and Related Antisense Biomolecules, Molecular Biology Intelligence Unit*; Janson, C. G., During, M. J., Eds.; Landes Bioscience/Kluwer Academic/Plenum Publishers: New York, 2006, p 59-86.

(42) Wickstrom, E.; Sauter, E. R.; Tian, X.; Rao, S.; Quin, W.; Thakur, M. L. *Braz. Arch. Biol. Technol.* **2002**, *45*, 57-59.

(43) Rusckowski, M.; Qu, T.; Chang, F.; Hnatowich, D. J. *Cancer* **1997**, *80*, 2699-2705, and references therein.

(44) Wang, Y.; Chang, F.; Zhang, Y.; Liu, N.; Liu, G.; Gupta, S.; Rusckowski, M.; Hnatowich, D. J. *Bioconjugate Chem.* **2001**, *12*, 807-816.

(45) Niklas, N.; Heinemann, F. W.; Hampel, F.; Clark, T.; Alsfasser, R. *Inorg. Chem.* **2004**, *43*, 4663-4673.

(46) Joseph, R.; Ramanujam, B.; Acharya, A.; Rao, C. P. *J. Org. Chem.* **2009**, *74*, 8181-8190.

(47) Niklas, N.; Alsfasser, R. *Dalton Trans.* **2006**, *26*, 3188-3199.

(48) Niklas, N.; Zahl, A.; Alsfasser, R. *Dalton Trans.* **2007**, *1*, 154-162.

(49) Sedlak, M.; Drabina, P.; Keder, R.; Hanusek, J.; Cisarova, I.; Ruzicka, A. *J. Organomet. Chem.* **2006**, *691*, 2623-2630.

(50) Marlin, D. S.; Cabrera, D. G.; Leigh, D. A.; Slawin, A. M. Z. *Angew. Chem. Int. Ed.* **2006**, *45*, 1385-1390.

(51) Best, M. D.; Anslyn, E. V. *Chem. Eur. J.* **2003**, *9*, 51-57.

(52) Niklas, N.; Heinemann, F. W.; Hampel, F.; Alsfasser, R. *Angew. Chem. Int. Ed.* **2002**, *41*, 3386-3388.

(53) Niklas, N.; Hampel, F.; Liehr, G.; Zahl, A.; Alsfasser, R. *Chem. Eur. J.* **2001**, *7*, 5135-5142.

(54) Hall, C. D.; Truong, T.-K.-U.; Tucker, J. H. R.; Steed, J. W. *Chem. Comm.* **1997**, *22*, 2195-2196.

(55) Cox, C.; Ferraris, D.; Murthy, N. N.; Lectka, T. *J. Am. Chem. Soc.* **1996**, *118*, 5332-5333.

(56) Pappalardo, S.; Bottino, F.; Finocchiaro, P.; Mamo, A. *J. Polym. Sci., Part A: Polym. Chem.* **1987**, *25*, 1793-1801. .

(57) Burth, R.; Stange, A.; Schaefer, M.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **1998**, *11*, 1759-1764.

(58) Kersebohm, T.; Kirin, S. I.; Metzler-Nolte, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2964-2968.

(59) Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. H. *J. Pept. Sci.* **1995**, *3*, 175-183.

(60) Banerjee, S. R.; Levadala, M. K.; Lazarova, N.; Wei, L.; Valliant, J. F.; Stephenson, K. A.; Babich, J. W.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **2002**, *41*, 6417-6425.

(61) Boyer, J. H. *J. Am. Chem. Soc.* **1951**, *73*, 5248-52.

(62) Babich, J. W.; Maresca, K. P. In *PCT Int. Appl. WO 2003077727* 2003, p 71.

(63) Mundwiler, S.; Candreia, L.; Häfliger, P.; Ortner, K.; Alberto, R. *Bioconjugate Chem.* **2004**, *15*, 195-202.

(64) Gasser, G.; Ott, I.; Metzler-Nolte, N. *J. Med. Chem.* **2010**, *54*, 3-25, and references therein.

(65) Brückmann, N. E.; Kögel, S.; Hamacher, A.; Kassack, M. U.; Kunz, P. C. *Eur. J. Inorg. Chem.* **2010**, 5063–5068.

(66) Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogebin, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598-8599.

(67) Alberto, R.; Egli, A.; Abram, U.; Hegetschweiler, K.; Gramlich, V.; Schubiger, P. A. *J. Chem. Soc. Dalton Trans.* **1994**, 2815-2820.

(68) Larsen, S.; Michelsen, K.; Pedersen, E. *Acta Chem. Scand.* **1986**, *A40*, 63-76.

(69) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.